

**WHAT IS CLAIMED IS:**

1. A method comprising:  
illuminating a sample to cause the sample to emit radiation, wherein the sample  
5 comprises deep tissue supporting a target compound, and wherein the emitted radiation  
comprises emission from the target compound and emission from one or more other  
components in the sample;  
spectrally filtering the emitted radiation with each of a plurality of different spectral  
weighting functions;  
10 storing an image of the spectrally filtered radiation for each of the spectral weighting  
functions; and  
processing the stored images to construct a deep tissue image of the sample in which  
signal from the other compounds is reduced relative to signal from the target compound.
- 15 2. The method of claim 1, wherein the sample comprising the deep tissue is a  
living organism.
3. The method of claim 2, wherein the living organism is an animal.
- 20 4. The method of claim 3, wherein the living organism is a mammal.
5. The method of claim 3, wherein the animal comprises a mouse, a zebrafish, or  
a human.
- 25 6. The method of claim 2, wherein the deep tissue is an internal organ of the  
living organism.
7. The method of claim 2, wherein the deep tissue lies within about 2 mm or  
more of the living organism.
- 30 8. The method of claim 1, wherein the deep tissue is subdermal tissue.

9. The method of claim 1, wherein the emission from the other components of the sample comprises autofluorescence from tissue overlying the deep tissue.

5 10. The method of claim 1, wherein the emission from the other components of the sample comprises autofluorescence from one or more layers of tissue in the sample different from a layer of tissue comprising the deep tissue.

11. The method of claim 1, wherein the target compound is a fluorescent probe  
10 bound to at least a portion of the deep tissue.

12. The method of claim 1, wherein the target compound is a quantum dot bound to at least a portion of the deep tissue.

15 13. The method of claim 1, wherein the target compound is a green fluorescent protein (GFP) bound to at least a portion of the deep tissue.

14. The method of claim 1, wherein the target compound is a yellow fluorescent protein (YFP) bound to at least a portion of the deep tissue.

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15. The method of claim 1, wherein the target compound is a red fluorescent protein (RFP) bound to at least a portion of the deep tissue.

16. The method of claim 1, wherein the emission from the target compound is  
25 fluorescence.

17. The method of claim 1, wherein at least some of the spectral weighting functions correspond to particular wavelength bands.

30 18. The method of claim 17, wherein all of the spectral weighting functions correspond to particular wavelength bands.

19. The method of claim 1, wherein at least some of the spectral weighting functions correspond to sinusoidal weightings of multiple wavelength bands.

5 20. The method of claim 1, wherein the spectral filtering comprises using a liquid-crystal, tunable optical filter.

21. The method of claim 1, wherein the spectral filtering comprises using an interferometric optical filter.

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22. The method of claim 1, wherein the spectral filtering comprises using a filter wheel containing a plurality of band pass filters.

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23. The method of claim 1, wherein each stored image comprises an intensity value for each of multiple pixels.

24. The method of claim 1, wherein processing the stored images comprises constructing the deep tissue image based on a weighted superposition of signals in the stored images.

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25. The method of claim 1, wherein processing the recorded images comprises constructing the deep tissue image based on the recorded images and at least one emission spectrum for the other components in the sample.

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26. The method of claim 25, wherein constructing the deep tissue image comprises calculating a remainder spectrum for each pixel in the set of stored images.

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27. The method of claim 25, wherein processing the recorded images comprises constructing the deep tissue image based on the recorded images, the at least one emission spectrum for the other components in the sample, and an emission spectrum for the target compound.

28. The method of claim 27, wherein constructing the deep tissue image comprises solving at least one component of a matrix equation in which one matrix is based on the stored images, and another matrix is based on the emission spectra.

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29. The method of claim 1, wherein processing the recorded images comprises constructing the deep tissue image based on the recorded images and an emission spectrum for the target compound.

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30. The method of claim 25, wherein constructing the deep tissue image comprises calculating a remainder spectrum for each pixel in the set of stored images.

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31. The method of claim 1, wherein the deep tissue supports multiple target compounds and processing the stored images comprises constructing a deep tissue image for each of the target compounds.

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32. The method of claim 31, wherein processing the recorded images comprises constructing the deep tissue images based on the recorded images and emission spectra for the target compounds.

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33. The method of claim 32, wherein processing the recorded images comprises constructing the deep tissue images based on the recorded images, the emission spectra for the target compounds, and at least one emission spectrum for the other components in the sample.

34. The method of claim 1, wherein the plurality of the different spectral weighting functions comprises at least four spectral weighting functions.

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35. A method comprising:  
providing a plurality of images of spectrally filtered radiation emitted from a sample in response to an illumination,

wherein the sample comprises deep tissue supporting a target compound,  
wherein the emitted radiation comprises emission from the target compound and  
emission from one or more other components in the sample, and  
wherein each image corresponds to a different spectral weighting function; and  
5 processing the images of the spectrally filtered radiation to construct a deep tissue  
image of the sample in which signal from the other compounds is reduced relative to signal  
from the target compound.

36. Apparatus comprising a computer readable medium which stores a program  
10 that causes a processor to:  
receive a plurality of images of spectrally filtered radiation emitted from a sample in  
response to an illumination,  
wherein the sample comprises deep tissue supporting a target compound,  
wherein the emitted radiation comprises emission from the target compound and  
15 emission from one or more other components in the sample, and  
wherein each image corresponds to a different spectral weighting function; and  
process the images of the spectrally filtered radiation to construct a deep tissue image of the  
sample in which signal from the other compounds is reduced relative to signal from the target  
compound.

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37. An apparatus comprising:  
a sample holder configured to hold a sample comprising deep tissue, wherein the deep  
tissue supports a target compound;  
an illumination source configured to illuminate the sample to cause it to emit  
25 radiation, wherein the emitted radiation comprises emission from the target compound and  
emission from one or more other components in the sample;  
an imaging system configured to image the emitted radiation to a detector;  
a tunable spectral filter configured to spectrally filter the emitted radiation with each  
of a plurality of different spectral weighting functions;  
30 a detector configured to store an image of the spectrally filtered radiation for each of  
the spectral weighting functions; and

a electronic processor configured to process the store images to construct a deep tissue image of the sample in which signal from the other compounds is reduced relative to signal from the target compound.

5           38.     The apparatus of claim 37, wherein the sample holder is configured to hold an animal.

          39.     The apparatus of claim 37, wherein the imaging system has a demagnification greater than or equal to 1.

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          40.     The apparatus of claim 37, wherein the imaging system is configured to image a field of view having a diagonal dimension greater than about 2 cm onto the detector.